REMARKS

Applicants thank the Examiner and the Examiner's supervisor for the courtesy extended to Applicants' attorney during the interview held May 23, 2005, in the above-identified application. During the interview, Applicants' attorney explained the presently-claimed invention and why it is patentable over the applied prior art, and discussed other issues raised in the Office Action. The discussion is summarized and expanded upon below.

The rejection of Claims 9-32 under 35 U.S.C. § 103(a) as unpatentable over JP8-165248 (JP '248) taken with the article by Elass-Rochard et al in *Infection and Immunity*, Vol. 66, No. 2, pp. 486-491, February 1998 (Elass-Rochard et al), is respectfully traversed.

As Applicants' attorney pointed out during the above-referenced interview, JP '248 is drawn to the use of peptides of lactoferrin (Lf), not Lf *per se*, and is thus irrelevant herein, since the presently-claimed invention is drawn to a method involving the administration of human-type lactoferrin (hLf) *per se*, not a peptide derived therefrom. Applicants hereby acknowledge the withdrawal of the above-pending ground of rejection, as reflected in the Interview Summary for the above-referenced interview.

The Interview Summary also indicates that there will be a new ground of rejection based on inherency. The argument below demonstrates that the presently-claimed invention is not inherent from the disclosure of Elass-Rochard et al alone. Indeed, as described in further detail below, Elass-Rochard et al discloses nothing more than *in vitro* results and thus cannot possibly inherently meet **any** method which involves *in vivo* administration of a medication.

The presently-claimed invention is drawn to a method for alleviating a symptom from lipopolysaccharide (LPS)-induced inflammation comprising administering to a person orally or parenterally an effective amount of hLf for a time and under conditions effective to alleviate said symptom. The symptom is accumulation of body fluid containing albumin at

the inflammation site (Claim 9); accumulation of albumin at the inflammatory site (Claim 15); decrease of albumin concentration in blood (Claim 21); or increase of neutrophils in blood (Claim 27).

Elass-Rochard et al discloses that "[t]he ability of hLF to form complexes with LPS (3, 11) and thus to inhibit the LPS-induced release of cytokines by mono-nuclear phagocytes (7, 27) makes it a potentially important molecule in the inflammatory response" (page 489, under "Discussion.") Specifically, Elass-Rochard et al discloses that hLF prevented the rhLBP-mediated binding of LPS to the CD14 receptors on the cells. "Maximal inhibition of LPS-cell interactions by hLF was raised when both hLf and rhLBP were simultaneously added to LPS or when hLf and LPS were mixed with cells 30 min. prior to the incubation with rhLBP" (page 486, under "Abstract.") Thus, the test in Elass-Rochard et al has confirmed that hLf inhibits binding of LPS to CD14 by competing with LPS, if hLf is present when a complex which is made by bonding of a LPS-binding protein, i.e., rhLBP, to LPS binds to CD14 (glycoprotein) present on the surface of macrophage.

However, Elass-Rochard et al does not show that binding of LPS to CD14 causes accumulation of body fluid containing albumin at the inflammatory site, accumulation of albumin thereat, decrease of albumin concentration in blood or increase of neutrophils in blood.

Cells capable of releasing endogenous mediating substances such as cytokinin are not limited to macrophage. In addition, there are many kinds of mediating substances and they each exhibit various actions or functions. Furthermore, the LPS-participating pathway in the living body includes a plurality of pathways other than the CD-14-dependent pathway. Indeed, Elass-Rochard et al concludes as follows:

> Further in vivo studies are needed to investigate whether Lf could directly overcome the LBP-mediated activation of cells

> in the host and modulate the CD14-independent LPS signalling pathways.

The Examiner finds, at pages 4-5 of the Office Action, as follows:

> On page 486, first paragraph, left column, [Elass-Rochard et al] states that it is known LPS are potent activators of the immune system. They stimulate host cells, mainly monocytes/macrophages and neutrophils, to produce endogenous mediators such as cytokines. On page 486, first paragraph, right column, the reference continues by stating that *in vivo*, hLf also regulates the release of TNF-α and protects mice against a lethal-dose of *E. coli*. **Thus, clearly showing that use of hLf increases blood neutrophils**.

(Emphasis added.)

In reply, and as Applicants' attorney pointed out during the above-referenced interview, the present invention uses hLf to alleviate increase of neutrophils in blood. This is just the reverse of what the Examiner finds.

The Examiner finds, at page 5 of the Office Action, as follows:

> On page 490, last paragraph, [Elass-Rochard et al] concludes by stating that Lf released from neutrophilic granules could neutralize the excess of LPS at the site of inflammation and protect the host against the excessive release of cytokines, and suggests that due to its high affinity for LPS, Lf could, *in vivo*, absorb small amounts of LPS.

In reply, Elass-Rochard et al does not disclose or suggest that administration of Lf can suppress all the releases of endogenous mediating substances such as cytokinin. This is apparent from the fact that Elass-Rochard et al concludes, as stated above:

> Further in vivo studies are needed to investigate whether Lf could directly overcome the LBP-mediated activation of cells in the host and modulate the CD14-independent LPS signalling pathways.

The Examiner finds, at page 6 of the Office Action, as follows:

> In regard to Applicant's allegation that [Elass-Rochard et al] discloses and suggests nothing to albumin exudation or increase of blood neutrophils, and thus nothing with regard to the presently claimed invention is unpersuasive. Contrary to

> Applicant's allegation, as acknowledged by Applicant on page 2, paragraph 2 in the instant specification, it is known in the art that during sepsis caused by gram-negative bacilli, decline in blood albumin concentration, decrease of lymphocytic leukocytes, and increase of neutrophil occur. Also, on page 4, Applicant acknowledges that bovine-type lactoferrin has been used to demonstrate an effect of alleviating various symptoms, which appear after infection. Thus, albumin exudation or increase of blood neutrophils at the inflammatory site, these are expected natural occurrence during inflammation whatever the cause of inflammation is. Therefore, in view of these and in view of the combined teachings of the prior art, particularly, the suggestion of the secondary reference of potential advantages of using hLf as discussed above, one of ordinary skill in the art would have been motivated at the time the invention was made to employ human-type lactoferrin for treatment or alleviating symptoms resulting from LPS-induced inflammation of human because of the expected species to species reaction.

In reply, the mechanisms of inflammation and immunity in the living body are very complicated, as stated previously. We repeat for purposes of emphasis that Elass-Rochard et al concludes:

> Further in vivo studies are needed to investigate whether Lf could directly overcome the LBP-mediated activation of cells in the host and modulate the CD14-independent LPS signal-pathways.

In addition, the albumin accumulation in the rats to which hLf was injected 18 hours prior to the administration of LPS was less than that in the counterparts of the rats to which hLf was injected only 15 minutes prior to the administration of LPS, as shown in Example 4 in the specification herein. See present Fig. 6. Accordingly, the competitive inhibition shown in Elass-Rochard et al cannot explain the present invention.

Further, Example 2 herein shows that bovine Lf showed no effect to alleviate albumin accumulation. This is also described in the poster presentation VI-13 (poster #29) of Abstracts in 5th International Conference on Lactoferrin, a copy of which is **submitted herewith**. In this paper, it is stated that an injection of anti rat serum IgG of TNF-α 15 min prior to LPS-injection did not affect the albumin extravasations.

To better appreciate the significance of the present invention, the Examiner should consider the following.

As described in the specification at paragraph [0003], Lf has been demonstrated in vitro to form a chelate with iron to mainly inhibit growth of E. coli, etc., and shows a bactericidal effect, as well as other pharmacological effects. As disclosed in paragraph [0004] of the specification, on the other hand, in inflammatory diseases, for example, ascites in peritonitis and bronchocavernous plasma exudation in pneumonitis are retained respectively to cause a decrease of physical strength of patients. Also severe exudation of neutrophils and tissue damage are induced in the inflammatory site. In sepsis caused by gram-negative bacilli, it is known that decline in blood albumin concentration, decrease of lymphocytic leukocytes, and increase of neutrophils occur and sometimes induce deterioration of the symptom, which in turn is developed into a systemic inflammatory reactive syndrome such as multiple organ failure, of which the prognosis is quite worse. In order to improve these symptoms resulting from inflammation, it has been attempted to administer a human-type albumin preparation into blood, but no sufficient alleviation effect for the symptoms has been recognized. In a case of serious invasion such as injury or a highly inflammatory state such as severe infection, it is considered that a large quantity of plasma water, would exude into the tissue because of sthenia of the endothelial permeability. In such a case, it has been reported that the use of a colloidal material such as an albumin preparation to alleviate the symptom has unexpectedly increased the risk of death. There is accordingly a demand for the development of a new agent to effectively alleviate these symptoms.

Thus, when inflammation occurs, endothelial permeability becomes high. Therefore, albumin in the blood exudes out of the blood vessel to the inflammatory site or region. Naturally, serum water also exudes and accumulates at the inflammatory site to cause

swelling, edema, accumulation of ascites, etc. For example, in the case of arthritis, accumulation of body fluid in the diseased knees causes pain.

The above-described albumin-exudation causes hypoalbuminemia that brings about lowering of immunity. Transfusion of an albumin preparation is employed as an expectant treatment for hypoalbuminemia. However, administered albumin exudes out of the blood vessel in an advanced state of endothelial permeability. In addition, transfusion of an albumin preparation is troublesome and is attended by risks of infection.

The present invention contributes to relief of pain in LPS-induced inflammation and/or contributes to the prevention or remediation of hypoalbuminemia by suppressing exudation of albumin out of the blood vessel.

In sum, Elass-Rochard et al discloses and suggests nothing with regard to albumin exudation or increase of blood neutrophils, and thus nothing with regard to the presently-claimed invention. Thus, the present invention, directed to a method for alleviating from LPS-inflammation by using hLf, (1) accumulation of body fluid containing albumin at the inflammatory site, (2) accumulation of albumin at the inflammatory site, (3) decrease of blood albumin concentration in blood, or (4) increase of neutrophils in blood, is not suggested by Elass-Rochard et al.

For all the above reasons, it is respectfully requested that no rejection over Elass-Rochard et al is sustainable, and thus such a rejection should not be made.

The objection to the specification is now moot in view of the above-discussed amendment. Accordingly, it is respectfully requested that the objection be withdrawn.

All of the presently-pending claims in this application are now believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

Respectfully submitted,

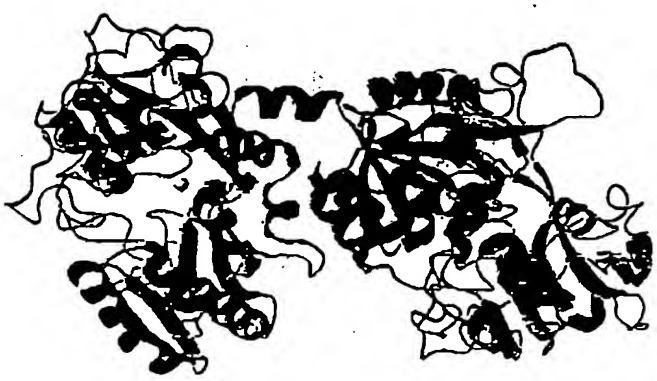
OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.
Norman F. Oblon

Harris A. Pitlick
Registration No. 38,779

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413 -2220
(OSMMN 08/03)
NFO/HAP/cja

5th International Conference on Lactoferrin: Structure, Function and Applications



May 4 - 9th, 2001

Banff, Alberta
Canada

5th International Conference on Lactoferrin: Structure, Function and Applications

May 4 - 9th, 2001
Banff, Alberta
Canada

LIST OF CONFERENCE SPONSORS

We gratefully acknowledge the generous support of the following sponsors:

GOLD SPONSORS

Morinaga Milk Company
National Institute of Environmental Health Sciences (NIEHS)
Alberta Heritage Foundation for Medical Research (AHFMR)
Agennix Inc.
Tatua Co-operative Dairy Company Limited
Borculo Domo Ingredients Biopole
DMV International Nutritionals

SILVER SPONSORS

DSM Food Specialties, Nutritional Ingredients
Pharming
Armor Proteins

BRONZE SPONSORS

Wyeth-Ayerst Int.

Abstracts

Session VI: Anti-inflammatory effects of Lactoferrin.

Oral Presentations: VI-1 to VI-6

Poster Presentations: VI-7 to VI-15:
(Poster # 23 - # 31)

VI-13 (Poster #29)

LACTOFERRIN AMELIORATES SEVERE ALBUMIN EXTRAVASATIONS AND NEUTROPHILIA IN LPS INJECTED NEONATAL RATS

Yajima M., Nakayama M., Aoyama Y., Yajima T.,Koide K., KuwataT.
Nutrition Science Institute, Meiji Milk Products Co., Ltd.

It is well known that peritoneal injection of lipopolysaccharide (LPS), a cell wall component of gram negative bacteria, induces severe septic-like inflammation followed by production of TNF-a in the plasma of rats, often resulting in death of the animal. Not only the TNF-a production, but also the death rate of the animals were reduced by a lactoferrin injection 18-20 hrs prior to the LPS injection. We observed that an i.p. injection of LPS induces acute inflammatory accumulation of albumin in ascitic fluid 5 hrs after LPS injection and neutrophilia in the blood 24 hrs after LPS injection. Therefore in the present study we examined whether lactoferrin has the ability to ameliorate the gross albumin extravasations and/or the neutrophilia resulting from plasma TNF-a induction.

The results show that an i.p. injection of human lactoferrin 18hrs prior to the LPS-injection reduced the mortality 24 hrs post LPS in neonatal rats. The LPS induced acute extravasations of albumin with accumulation of ascitic fluid 5 hrs after LPS-injection, and neutrophilia in the blood 24 hrs post LPS injection were also ameliorated. An injection of anti rat serum IgG of TNF-a 15 min prior to LPS-injection did not affect the albumin extravasations. Separate injection of two commercially available bovine lactoferrins prior to LPS injection decreased TNF-a in the plasma to the same degree as with human lactoferrin, but did not ameliorate the albumin extravasations .

In conclusion, the present study demonstrated that lactoferrin has the ability to ameliorate LPS-induced septic like inflammatory responses such as albumin extravasations and neutrophilia, which may not involve mediation of plasma TNF-a elevation.